STAFF SUMMARY FOR JUNE 21-22, 2017

11. COMMERCIAL USE AND POSSESSION OF NATIVE RATTLESNAKES

Today's Item **Information** ☐ **Action** ☒

Authorization to publish notice of intent to add a section to allow for commercial use of native rattlesnakes.

Summary of Previous/Future Actions

- **Today's notice hearing** **Jun 21-22, 2017; Smith River**
- Discussion/adoption hearing Oct 11-12, 2017; Atascadero

Background

FGC received a petition in 2015 to amend existing regulations or adopt new regulations that would allow for the commercial use of native rattlesnakes to develop antivenom, vaccines, and other therapeutic agents. FGC approved the petition request at its Feb 11, 2016 meeting in Sacramento and forwarded it to DFW for evaluation.

DFW staff met with the petitioners during 2016 to gather additional information. The petitioners had initially proposed using "nuisance" snakes collected by rattlesnake removal businesses for this purpose, as well as raising the possession limit on native rattlesnakes for aversion trainers. However, those proposals would have required additional public outreach and scoping of affected businesses that would have greatly delayed the development of the new regulations. Therefore, with the petitioners' consent, DFW narrowed the scope of the regulatory proposal to address only commercialized use of native rattlesnakes for venom extraction in conjunction with research and development of biomedical and therapeutic agents. In addition, DFW added propagation of native rattlesnakes at the request of the petitioners. The proposed regulations would authorize limited commercial use of native rattlesnakes for the purposes of developing biomedical and therapeutic products that will benefit humans and domestic animals.

The proposed Section 42 regulation will allow California businesses to develop and sell regionally specific antivenom, vaccines, and therapeutic agents derived from native rattlesnake venom that would benefit human, pet, and livestock health. The new permit is structured to allow for businesses that seek to maintain live native rattlesnake species for venom extraction to develop and sell therapeutic products from the native rattlesnake venom, or businesses that only intend to develop and sell therapeutic products from the native rattlesnake venom.

In addition, it is necessary to make minor amendments to Sections 43, 651, and 703 to provide consistency and clarity with the proposed Section 42 (see Exhibit 2).

Significant Public Comments (N/A)

Recommendation

FGC staff: Authorize publication of the notice as recommended by DFW

Exhibits

1. DFW memo, received May 26, 2017
2. Initial statement of reasons
3. Cates et al., American Journal of Veterinary Research, 2015, Mar: 76(3):272-79, document relied upon

Motion/Direction

Moved by _____ and seconded by _____ that the Commission authorizes publication of a notice of its intent to add Section 42, amend sections 43, 651 and 703, related to commercial use of rattlesnakes for biomedical and therapeutic purposes.

State of California
Department of Fish and Wildlife

Memorandum

Date: May 18, 2017

To: Valerie Termini
Executive Director
Fish and Game Commission

From: Charlton H. Bonham
Director

Subject: **Agenda Item for the June 21-22, 2017, Fish and Game Commission Meeting Request for Notice Authorization to Add Section 42, Title 14, California Code of Regulations (CCR), and Amend sections 43, 651 and 703, Title 14, CCR, RE: Commercial Use and Possession of Native Rattlesnakes for Biomedical and Therapeutic Purposes**

Attached, please find the Initial Statement of Reasons to add Section 42 and subsection (a)(2) of Section 703 to Title 14, CCR and to amend subsection (c) of Section 43 and subsection (a) of Section 651, Title 14, CCR.

The Fish and Game Commission (Commission) received a petition in 2015 (Petition No. 2015-004) to amend existing regulations or adopt new regulations that would allow for the commercial use of native rattlesnakes to develop antivenom, vaccines, and other therapeutic agents. The Commission approved the petition request at its February 11, 2016 meeting in Sacramento and forwarded it to the Department of Fish and Wildlife (Department) for evaluation.

Department staff met with the petitioners during 2016 to gather additional information. The petitioners had initially proposed using "nuisance" snakes collected by rattlesnake removal businesses for this purpose, as well as raising the possession limit on native rattlesnakes for aversion trainers. However, those proposals would have required additional public outreach and scoping of affected businesses that would have greatly delayed the development of the new regulations. Therefore, with the petitioners' consent, the Department narrowed the scope of the regulatory proposal to address only commercialized use of native rattlesnakes for venom extraction in conjunction with research and development of biomedical and therapeutic agents. In addition, the Department added propagation of native rattlesnakes at the request of the petitioners.

The Commission has the statutory authority to adopt regulations for the commercial use of native reptiles pursuant to Fish and Game Code Section 5061. The current regulatory proposal would authorize limited commercial use of native rattlesnakes for the purposes of developing biomedical and therapeutic products that will benefit humans and domestic animals.

It establishes a new Commercial Native Rattlesnake Permit Application (Form DFW 1044) and a new Commercial Native Rattlesnake Record (Form DFW 1044A) issued by the Department.

If you have any questions regarding this item, please contact T.O. Smith, Chief, Wildlife Branch, by telephone at (916) 445-3555 or by e-mail at Timothy.Smith@wildlife.ca.gov. The public notice should identify Laura Patterson, Senior Environmental Scientist in the Nongame Wildlife Program, as the Department's point of contact for this rulemaking. Ms. Patterson can be reached at (916) 341-6981 or by e-mail at Laura.Patterson@wildlife.ca.gov.

Attachments

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STATE OF CALIFORNIA
FISH AND GAME COMMISSION
INITIAL STATEMENT OF REASONS FOR REGULATORY ACTION
(Pre-publication of Notice Statement)

Add Section 42 and subsection (a)(2) of Section 703, and
Amend subsection (c) of Section 43 and subsection (a) of Section 651,
Title 14, California Code of Regulations
Re: Commercial Use and Possession of Native Rattlesnakes
for Biomedical and Therapeutic Purposes

I. Date of Initial Statement of Reasons: April 12, 2017

II. Dates and Locations of Scheduled Hearings:

(a) Notice Hearing: Date: June 21, 2017
Location: Smith River

(b) Discussion and Adoption Hearing: Date: October 11, 2017
Location: Atascadero

III. Description of Regulatory Action:

(a) Statement of Specific Purpose of Regulation Change and Factual Basis for Determining that Regulation Change is Reasonably Necessary:

The Fish and Game Commission (Commission) received a petition in 2015 to amend existing regulations or adopt new regulations that would allow for the commercial use of native rattlesnakes to develop antivenom, vaccines, and other therapeutic agents. The Commission approved the petition request at its February 11, 2016 meeting in Sacramento and forwarded it to the Department of Fish and Wildlife (Department) for evaluation.

Department staff met with the petitioners during 2016 to gather additional information. The petitioners had initially proposed using “nuisance” snakes collected by rattlesnake removal businesses for this purpose, as well as raising the possession limit on native rattlesnakes for aversion trainers. However, those proposals would have required additional public outreach and scoping of affected businesses that would have greatly delayed the development of the new regulations. Therefore, with the petitioners’ consent, the Department narrowed the scope of the regulatory proposal to address only commercialized use of native rattlesnakes for venom extraction in conjunction with research and development of biomedical and therapeutic agents. In addition, the Department added propagation of native rattlesnakes at the request of the petitioners.

The Commission has the statutory authority to adopt regulations for the commercial use of native reptiles pursuant to Fish and Game Code Section 5061. Currently, there are only two authorized commercial activities in California: captive propagation and sale of three species of snakes, which is allowed under Section 43, and wild collection and sale of native reptiles by Biological Supply Houses, which is allowed under Section 651.

According to the California Poison Control System, over 300 rattlesnake bites are reported in the state each year. According to the National Institutes of Health, approximately 7,000-8,000 people receive venomous bites in the United States and about 5 people die. While exact numbers are unavailable, it has been estimated that well over 100,000 domesticated animals are bitten annually in the United States by venomous snakes, sometimes resulting in death. Rattlesnake bites are known to cause serious tissue, muscle, liver, and neurological damage. The composition of rattlesnake venom differs by species, and in some cases by location within the species. For example, Southern Pacific Rattlesnake (*Crotalus oreganus helleri*) venom has unique properties that differ across its range. Antivenom and vaccines that are derived from different species of rattlesnakes than the species that inflicted the bite are less effective, and sometimes not effective at all, in treatment of the bite. The currently available rattlesnake vaccine for domestic animals is derived from Western Diamondback Rattlesnake (*Crotalus atrox*) venom. A study in the American Journal of Veterinary Medicine (Cates et al. 2015) found this vaccine improved survival rate and survival time after envenomation from Western Diamondback Rattlesnakes. However, while it may offer some limited protection against Northern Pacific Rattlesnake (*Crotalus oreganus oreganus*) venom, it did not provide significant protection against Southern Pacific Rattlesnake venom.

Amendments to existing commercially authorized activities pursuant to Sections 43 and 651 are impractical. Section 43 pertains to the production of captive born reptiles for the purpose of selling them in the pet trade and has no application to the commercialization of rattlesnake venom or products derived from venom. Section 651 is restricted to the sale of native reptiles and amphibians collected from the wild to scientific and educational institutions by owners of biological supply houses that have been issued a permit from the Department. Therefore, to advance public and domestic animal health and safety, a new regulation is being proposed (Section 42) to address the need for regionally specific antivenom, vaccines, and other venom-derived therapeutic agents, that are effective against the bites from native rattlesnakes and provide other biomedical benefits. This new regulation would authorize commercial development of these products by California businesses under a permit issued by the Department.

Existing Regulations

The text of Section 42 was repealed in January 2002, but the title and note are still listed in Title 14, California Code of Regulations (CCR). Section 43 contains

regulations for the captive propagation of native reptiles and sale of three species of native snakes for the pet trade. Section 651 regulations specify the wild collection and sale of native reptiles by Biological Supply Houses.

Proposed Regulations
The proposed new Section 42 regulation will allow California businesses to develop and sell regionally specific antivenom, vaccines, and other therapeutic agents derived from native rattlesnake venom. These products would benefit livestock, pet, and eventually, human health. The new permit will allow:

1. Businesses to maintain live native rattlesnake species for the purposes of venom extraction and the development and sale of therapeutic products derived from native rattlesnake venom, or
2. Businesses to develop and sell therapeutic products derived from commercially obtained native rattlesnake venom.

In addition, it is necessary to make minor amendments to sections 43, 651, and 703 to provide consistency and clarity with the proposed Section 42.

Section 42

Subsection (a) of Section 42 details the activities allowed under a commercial native rattlesnake permit issued by the Department. This subsection is necessary to provide the context for the purpose of the regulation and to specify the activities that would be authorized under a permit issued pursuant to the regulation.

Subsection (b) of Section 42 specifies that this regulation does not supersede any other federal, state, or local laws regulating or prohibiting possession of native rattlesnakes or the activities authorized under a commercial native rattlesnake permit. This subsection is necessary to ensure consistency with other laws and to clarify that this regulation does not supplant existing or future restrictions on the possession and use of native rattlesnakes by other jurisdictions.

Subsection (c) of Section 42 lists the species of native rattlesnakes that may be used under this regulation. This subsection is necessary to make it explicit that all currently recognized species of native rattlesnakes, their subspecies and taxonomic successors, are allowed to be used for the purposes of this regulation with the exception of the Red Diamond Rattlesnake (*Crotalus ruber*), which is a California Species of Special Concern.

Subsection (d) of Section 42 specifies requirements for the permit application, fees associated with the application, duration of permit, and qualification requirements. A separate permit is proposed for each facility housing native

rattlesnake species or creating products from venom extracted from native rattlesnake species. The qualification requirements differ depending on whether the applicant plans to house live native rattlesnakes in their facility as follows:

1. If the applicant proposes to house live native rattlesnake species for the purposes of developing therapeutic products from venom, minimum experience and animal husbandry qualifications are proposed. A resume demonstrating a minimum of 1,000 hours experience with captive husbandry of snakes and 200 hours working directly with captive rattlesnakes or other venomous snakes within five years of the date of application is required. The Department believes these are the minimum amounts of time necessary for individuals to obtain the skills needed to competently, and safely handle native rattlesnakes. In addition, an original, signed letter of reference is required as documentation that the experience requirements have been met. A statement of purpose for maintaining native rattlesnakes and a Written Emergency Action Plan are also required. Proof of minimum age (18 years) is also required.
2. If the applicant proposes only to develop therapeutic products from venom, the animal husbandry and Emergency Action Plan requirements no longer apply. A resume and an original, signed letter of reference documenting the applicant's experience are required. A statement of purpose for the planned use of the venom and proof of minimum age (18 years) are also required.

This subsection is necessary to inform potential applicants of the application process, minimum qualifications, and fees involved in obtaining and maintaining a permit issued pursuant to this section. The proposed regulation establishes a new Commercial Native Rattlesnake Application (Form DFW 1044 (New 4/2017)), which is incorporated by reference herein.

Subsection (e) of Section 42 describes the general conditions associated with possessing a permit pursuant to this Section, including agreeing to random inspections, ability to transfer or exchange rattlesnakes among permittees, prohibition of release into the wild, and conditions under which applications will be denied or permits will be revoked. This subsection is necessary to inform potential applicants of the terms and conditions associated with possessing a permit pursuant to this section.

Subsection (f) of Section 42 describes the humane care and treatment that permittees must provide to native rattlesnakes possessed under this regulation. This subsection specifies requirements for enclosure size, substrate, and cleanliness; appropriate food and water; pest control; and observation and handling. This subsection will align the new regulations with the existing requirements in subsection 43(g). This subsection is necessary to inform applicants of the minimum care and treatment standards required to obtain a permit pursuant to this regulation and for consistency with the requirements of

subsection 43(g).

Subsection (g) of Section 42 describes the requirement for each facility to maintain an Emergency Action Plan and the minimum contents of that plan in the event a bite, escape, or emergency evacuation. This subsection is necessary because permitted facilities may be housing large numbers of venomous snakes which may result in a public health and safety issue. The Emergency Action Plan will prepare the permittee and its employees in responses to accidental escapes and bites and ensure appropriate equipment is stored on site. It will also ensure appropriate agencies are notified in a timely manner of an escape or any serious injury or death of a person bitten by a native rattlesnake possessed under a commercial native rattlesnake permit.

Subsection (h) of Section 42 describes the records a permittee must maintain while operating under a permit pursuant to this section and the duration the records must be kept and made available to the Department. This subsection is necessary to ensure that the permittee is complying with the terms of the permit and regulation. The proposed regulation establishes a new Commercial Native Rattlesnake Record (Form DFW 1044A (New 4/2017)), which is incorporated by reference herein.

Subsection (i) of Section 42 describes the annual reporting requirements under the regulation. This subsection is necessary to inform applicants that the records maintained under subsection (h) must be submitted to the Department on an annual basis.

Subsection (j) of Section 42 describes the terms of shipping live native rattlesnakes under the authority of this regulation and clarifies that this regulation does not supersede any federal, state, local, or shipping entity's rules regarding shipment of live rattlesnakes. This subsection is necessary to ensure proper notification to postal workers, documentation to law enforcement that the native rattlesnakes are being shipped legally under the authority of this regulation, and to ensure this regulation does not conflict with any other jurisdiction's rules or regulations regarding shipping native rattlesnakes.

Subsection (c) of Section 43

Subsection (c) of Section 43 restricts the sale, possession, transportation, importation, exportation, and propagation of native reptiles for commercial purposes to subsection 40(f) and the regulations contained within Section 43. To ensure consistency with the new regulations, this subsection needs to be amended to allow an exception for entities permitted through Section 42.

Subsection (a) of Section 651

Subsection (a) of Section 651 limits the sale of native reptiles and amphibians to

scientific or educational institutions to biological supply houses that operate under a permit issued by the Department. Confusion regarding whether these institutions can also develop commercial products from the native reptiles and amphibians requires the addition of clarifying language proposed in this amendment. The proposed language explicitly states that persons who hold a valid commercial native rattlesnake permit issued by the Department and commercial developers of biomedical or therapeutic agents shall be considered scientific and educational institutions for the purposes of this Section.

Subsection (a)(2) of Section 703

Subsection (a)(2) of Section 703 provides the forms and fees associated with the Commercial Native Rattlesnake Permit.

(b) Authority and Reference Sections from Fish and Game Code for Regulation:

Authority: Section 5061, Fish and Game Code. Section 597, Penal Code. Sections 11503 and 11506, Government Code.

Reference: Sections 5060 and 5061, Fish and Game Code. Section 597, Penal Code. Sections 11503 and 11506, Government Code.

(c) Specific Technology or Equipment Required by Regulatory Change:

None.

(d) Identification of Reports or Documents Supporting Regulation Change:

Cates, C.C., E.V. Valore, G.W. Lawson, and J.G. McCabe. 2015. Comparison of the protective effect of a commercially available western diamondback rattlesnake toxoid vaccine for dogs against envenomation of mice with western diamondback rattlesnake (*Crotalus atrox*), northern Pacific rattlesnake (*Crotalus oreganus oreganus*), and southern Pacific rattlesnake (*Crotalus oreganus helleri*) venom. American Journal of Veterinary Research 76(3):272-279.

(e) Public Discussions of Proposed Regulations Prior to Notice Publication:

No public meetings are being held prior to the notice publication. The 45-day comment period provides adequate time for review of the proposed amendments.

IV. Description of Reasonable Alternatives to Regulatory Action:

(a) Alternatives to Regulation Change:

The Department evaluated amending Section 43 “Captive Propagation and Commercialization of Native Reptiles” to include native rattlesnakes in subsection (c). This alternative was rejected due to the desire to maintain a narrow scope on the allowable commercial use of native rattlesnakes in the new regulation (i.e., solely for the development and sale of therapeutic products). Because the original purpose of Section 43 was to authorize propagation of select species for the pet trade, it is necessary to keep commercial use of native rattlesnakes in a separate section to avoid confusion and the unintended creation of a commercial market for native rattlesnakes.

(b) No Change Alternative:

Under the no change alternative, no commercial production of antivenom, vaccines, or other biomedical and therapeutic agents derived from native rattlesnakes could legally occur in California.

(c) Consideration of Alternatives:

In view of information currently possessed, no reasonable alternative considered would be more effective in carrying out the purpose for which the regulation is proposed, would be as effective and less burdensome to affected private persons than the proposed regulation, or would be more cost effective to affected private persons and equally effective in implementing the statutory policy or other provision of law.

V. Mitigation Measures Required by Regulatory Action:

The proposed regulatory action is not expected to have a significant effect on the environment; therefore, no mitigation measures are needed.

VI. Impact of Regulatory Action:

The potential for significant statewide adverse economic impacts that might result from the proposed regulatory action has been assessed, and the following initial determinations relative to the required statutory categories have been made:

(a) Significant Statewide Adverse Economic Impact Directly Affecting Businesses, Including the Ability of California Businesses to Compete with Businesses in Other States:

The proposed action will not have a significant statewide adverse economic impact directly affecting business, including the ability of California businesses to compete with businesses in other states. It

establishes the ability for California companies to compete with out-of-state companies in the development and sale of pharmaceutical products derived from native rattlesnakes.

(b) Impact on the Creation or Elimination of Jobs Within the State, the Creation of New Businesses or the Elimination of Existing Businesses, or the Expansion of Businesses in California; Benefits of the Regulation to the Health and Welfare of California Residents, Worker Safety, and the State's Environment:

The Commission does not anticipate significant impacts on the creation or elimination of jobs, the creation of new business, the elimination of existing businesses or the expansion of businesses in California due to the limited number of anticipated permit applications.

The Commission anticipates benefits to the health and welfare of California residents through the development of improved therapeutic agents to treat rattlesnake bites in pets and domestic livestock.

The Commission does not anticipate any non-monetary benefits to worker safety.

(c) Cost Impacts on a Representative Private Person or Business:

The Commission estimates that a representative private person or business would necessarily incur $815 in permitting and inspection costs in the first year and $113 in annual costs in reasonable compliance with the proposed action.

(d) Costs or Savings to State Agencies or Costs/Savings in Federal Funding to the State:

The Commission anticipates revenue to recover the Department's administrative costs from initial inspections and permit fees for the first year from each business and annual renewal fees thereafter. The proposed action will not affect any other State Agency.

(e) Nondiscretionary Costs/Savings to Local Agencies:

None

(f) Programs Mandated on Local Agencies or School Districts:

None.

(g) Costs Imposed on Any Local Agency or School District that is Required to be Reimbursed Under Part 7 (commencing with Section 17500) of Division 4, Government Code:

None.

(h) Effect on Housing Costs:

None.

VII. Economic Impact Assessment:

(a) Effects of the Regulation on the Creation or Elimination of Jobs Within the State:

Due to the limited number of expected applicants, the regulation has the potential to create a small number of jobs in the State. The proposed regulation should not eliminate any jobs.

(b) Effects of the Regulation on the Creation of New Businesses or the Elimination of Existing Businesses Within the State:

The regulation is expected to provide new business opportunities within the State.

(c) Effects of the Regulation on the Expansion of Businesses Currently Doing Business Within the State:

None.

(d) Benefits of the Regulation to the Health and Welfare of California Residents:

Allowing for limited collection and possession of native rattlesnakes as described in Section 42 is expected to result in more effective and cheaper antivenom and vaccines as well as other therapeutic agents**.**

(e) Benefits of the Regulation to Worker Safety:

None.

(f) Benefits of the Regulation to the State's Environment:

None.

(g) Other Benefits of the Regulation:

None.

Informative Digest/Policy Statement Overview

The Fish and Game Commission (Commission) received a petition in 2015 to amend existing regulations or adopt new regulations that would allow for the commercial use of native rattlesnakes to develop antivenom, vaccines, and other therapeutic agents. The Commission approved the petition request at its February 11, 2016 meeting in Sacramento and forwarded it to the Department of Fish and Wildlife (Department) for evaluation. Department staff met with the petitioners during 2016 to gather additional information. The petitioners had initially proposed using "nuisance" snakes collected by rattlesnake removal businesses for this purpose, as well as raising the possession limit on native rattlesnakes for aversion trainers. However, those proposals would have required additional public outreach and scoping of affected businesses that would have greatly delayed the development of the new regulations. Therefore, with the petitioners' consent, the Department narrowed the scope of the regulatory proposal to address only commercialized use of native rattlesnakes for venom extraction in conjunction with research and development of biomedical and therapeutic agents. In addition, the Department added propagation of native rattlesnakes at the request of the petitioners.

The Commission has the statutory authority to adopt regulations for the commercial use of native reptiles pursuant to Fish and Game Code Section 5061. Currently, there are only two authorized commercial activities in California: captive propagation and sale of three species of snakes, which is allowed under Section 43, and wild collection and sale of native reptiles by Biological Supply Houses, which is allowed under Section 651.

Venom from rattlesnakes differs by species, and in some cases by location within the species. For example, Southern Pacific Rattlesnake (*Crotalus oreganus helleri*) venom has unique properties that differ across its range. Antivenom and vaccines that are derived from different species of rattlesnakes than the species that inflicted the bite are less effective, and sometimes not effective at all, in treatment of the bite. Currently, the only way antivenom, vaccines, and therapeutic agents can be derived from native rattlesnakes in California is through non-commercial research and development through a valid Scientific Collecting Permit pursuant to Section 650. However, Biological Supply Houses can collect native rattlesnakes and sell them to out-of-state scientific and educational facilities that develop and sell these products.

Existing Regulations

The text of Section 42 was repealed in January 2002, but the title and note are still listed in Title 14, Code of Regulations (CCR). Section 43 contains regulations for the captive propagation of native reptiles and sale of three species of native snakes. Section 651 regulations specify the wild collection and sale of native reptiles by Biological Supply Houses.

Proposed Regulations

The proposed Section 42 regulation will allow California businesses to develop and sell regionally specific antivenom, vaccines, and therapeutic agents derived from native

rattlesnake venom that would benefit human, pet, and livestock health. The new permit is structured to allow for:

1. Businesses which seek to maintain live native rattlesnake species for venom extraction and develop and sell therapeutic products from the native rattlesnake venom, or
2. Businesses which only intend to develop and sell therapeutic products from the native rattlesnake venom.

In addition, it is necessary to make minor amendments to Sections 43, 651, and 703 to provide consistency and clarity with the proposed Section 42.

Subsection (a) of Section 42 details the activities that the activities that allowed with a commercial native rattlesnake permit issued by the Department.

Subsection (b) of Section 42 specifies that this regulation does not supersede any other federal, state, or local laws regulating or prohibiting possession of native rattlesnakes or the activities authorized under a commercial native rattlesnake permit.

Subsection (c) of Section 42 lists the species of native rattlesnakes that may be used under this regulation.

Subsection (d) of Section 42 specifies regulations for the permit application, fees, duration of permit, and qualification requirements, such as minimum qualifications, letter of reference, statement of purpose, an emergency action plan, an initial inspection and minimum age. A separate permit is proposed for each facility housing native rattlesnake species or creating products from venom extracted from native rattlesnake species. The proposed regulation establishes a new Commercial Native Rattlesnake Application (Form DFW 1044 (New 4/2017)), which is incorporated by reference herein.

Subsection (e) of Section 42 describes the general conditions associated with possessing a permit pursuant to this section, including agreeing to random inspections, ability to transfer or exchange rattlesnakes among permittees, prohibition of release into the wild, and conditions under which applications will be denied or permits will be revoked.

Subsection (f) of Section 42 describes the humane care and treatment that permittees must provide to native rattlesnakes possessed under this regulation. It includes requirements on enclosure size, substrate, and cleanliness; appropriate food and water; pest control; and observation and handling.

Subsection (g) of Section 42 describes the requirement for each facility to maintain an Emergency Action Plan and the minimum contents of that plan in the event of a bite, escape, or emergency evacuation.

Subsection (h) of Section 42 describes the records a permittee must maintain while operating under a permit pursuant to this section and the duration the records must be kept and made available to the department. The proposed regulation establishes a new Commercial Native Rattlesnake Record (Form DFW 1044A (New 4/2017)), which is incorporated by reference herein.

Subsection (i) of Section 42 describes the annual reporting requirements under the regulation.

Subsection (j) of Section 42 describes the terms of shipping live native rattlesnakes under the authority of this regulation and clarifies that this regulation does not supersede any federal, state, local, or shipping entity's rules regarding shipment of live rattlesnakes.

Subsection (c) of Section 43 restricts the sale, possession, transportation, importation, exportation, and propagation of native reptiles for commercial purposes except as provided in subsection 40(f) and the species identified within Section 43. To ensure consistency with the new regulation, this amendment adds an exception for entities permitted through Section 42.

Subsection (a) of Section 651 limits the sale of native reptiles and amphibians to scientific or educational institutions to biological supply houses that operate under a permit issued by the Department. This proposed amendment states that persons who hold a valid commercial native rattlesnake permit issued by the department and commercial developers of biomedical or therapeutic agents shall be considered scientific and educational institutions for the purposes of this section.

Subsection (a)(2) of Section 703 specifies the forms and fees associated with the Commercial Native Rattlesnake Permit.

Benefits of the regulations

Allowing for limited collection and possession of native rattlesnakes as described in Section 42 is expected to result in more effective and cheaper antivenom and vaccines as well as other therapeutic agents.

Consistency with State and Federal Regulations

Article IV, section 20 of the State Constitution specifies that the Legislature may delegate to the Fish and Game Commission such powers relating to the protection and propagation of fish and game as the Legislature sees fit. The Legislature has delegated to the Commission the power to regulate commercial take of native reptiles (Fish & Game Code, §5061). The Commission has reviewed its own regulations and finds that the proposed regulations are neither inconsistent nor incompatible with existing state regulations. The Commission has searched the California Code of Regulations and finds no other state agency regulations pertaining to native rattlesnakes. Further, the

Commission has determined that the proposed regulations are neither incompatible nor inconsistent with existing federal regulations.

Regulatory Language

Add Section 42, to Title 14, CCR:

Section 42. ~~Protected Reptiles~~Commercial Use and Possession of Native Rattlesnakes for Biomedical and Therapeutic Purposes.

(a) Except as otherwise provided in these regulations, it shall be unlawful for persons without a valid commercial native rattlesnake permit issued by the department to:

(1) possess, purchase, propagate, exchange, or transport native rattlesnakes for commercialized venom extraction; or

(2) sell, import, or export native rattlesnake venom or products derived from native rattlesnake venom for commercial purposes.

(b) Consistency with Federal, State, and Local Laws.

A permit issued pursuant to this section does not supersede any federal, state, or local law regulating or prohibiting native rattlesnakes or the activities authorized in a commercial native rattlesnake permit.

(c) Authorized Native Rattlesnake Species.

A commercial native rattlesnake permit may be issued pursuant to this section for the following native rattlesnake species, including their subspecies and taxonomic successors:

(1) Western diamond-backed rattlesnake (*Crotalus atrox*),

(2) Mohave rattlesnake (*Crotalus scutulatus*),

(3) Western rattlesnake (*Crotalus oreganus*),

(4) Speckled rattlesnake (*Crotalus mitchellii*),

(5) Sidewinder (*Crotalus cerastes*), and

(6) Panamint rattlesnake (*Crotalus stephensi*).

(d) Permit Application and Fees.

(1) Application for a permit shall be made on the application form specified in Section 703. Application forms are available on the department's website at www.wildlife.ca.gov. The application form shall be completed in its entirety and submitted with the permit and nonrefundable inspection fees as specified in Section 703.

(2) Duration of Permit. Permits issued under this section shall be valid from January 1 through December 31 each year, or if issued after the beginning of that term, for the remainder thereof. Applications for renewal must be received by the department no later than November 1.

(3) Permitted facilities. A person shall obtain a separate commercial native rattlesnake permit for each facility housing native rattlesnake species or creating products from venom extracted from native rattlesnake species described in subsection (c) for purposes described in subsection (a).

(4) Qualifications. The following information and documents shall accompany an application for each new permit or renewal unless specified as exempt or as specifically required:

(A) For an application that proposes housing live native rattlesnake species and will

develop products derived from venom extracted from native rattlesnake species:
1. A resume that provides the dates and description of an applicant's or their employee's experience working with venomous snakes and husbandry of captive snakes, demonstrating the following qualifications:
a. Possess a minimum of 1000 hours experience with captive husbandry of snakes within five (5) years of the date of application; and
b. Possess a minimum of 200 hours of experience working with captive rattlesnakes or other venomous snakes within five (5) years of the date of application.
2. A letter of reference from an expert in venomous snake captive husbandry and research, dated within five (5) years of the date of application, on letterhead stationery with an original signature signed in ink by the owner or operator of a facility where the applicant or their employee gained his/her experience. The letter shall provide the printed name of the owner or operator and detailed information regarding the quality and extent of the applicant's or their employee's knowledge and experience related to the permit requested.
3. A statement of purpose describing in detail the planned uses for the species.
4. A written Emergency Action Plan as specified in subsection (g).
5. An initial inspection is required for new permits prior to the permit being issued.
6. Proof that the applicant is at least 18 years of age at the time of application.
(B) For an application that does not propose housing live native rattlesnakes and will only develop products derived from venom extracted from native rattlesnake species:
1. A resume that provides the dates and description of an applicant's or their employee's experience researching and creating products from venom extracted from native rattlesnake species.
2. A letter of reference from an expert in venomous snake research, dated within five (5) years of the date of application, on letterhead stationery with an original signature signed in ink by the owner or operator of a facility where the applicant or their employee gained his/her experience. The letter shall provide the printed name of the owner or operator and detailed information regarding the quality and extent of the applicant's or their employee's knowledge and experience related to the permit requested
3. A statement of purpose describing in detail the planned uses for the venom.
4. Proof that the applicant is at least 18 years of age at the time of application.

(e) General Conditions.

(1) Inspections. The department may enter the facilities of any permittee where native rattlesnakes are housed, or reasonably may be housed, at any reasonable hour to inspect the animals and their enclosures and to inspect, audit or copy records required by this section.
(A) The department may deny the issuance of, or immediately suspend, the permit of a permittee who refuses to allow inspection of a facility, permit, book, or other record required to be kept by the permittee. A refusal to allow inspection may be inferred if, after reasonable attempts by the department, the permittee does not make the facility,

permit, book, or other record available for inspection. The department may reinstate a permit suspended pursuant to this subsection if the permittee allows the department to inspect the facility, permit, book, or other record.

(2) Native rattlesnakes possessed pursuant to this section may be transferred to or exchanged with a person with a valid commercial native rattlesnake permit. The receiving permittee may be charged only to recover actual transportation and shipping costs.

(3) Native rattlesnakes which have been in captivity, including wild-caught and captive-bred individuals or offspring, shall not be released into the wild.

(4) Denial. The department shall deny a commercial native rattlesnake permit initial application or renewal application for any applicant who fails to comply with any provision in this regulation, and may deny an initial application or renewal application for any applicant who violates the Fish and Game Code, Title 14 regulations, any term or condition of a commercial native rattlesnake permit, or any other state or federal statute or regulation pertaining to wildlife or animal cruelty. Within 30 calendar days of a denial, an applicant may submit a written request for a hearing before the commission to show cause why his/her permit should be issued.

(5) Revocation. Any permit issued pursuant to these regulations may be suspended or revoked at any time by the department as described below.

(A) For a permittee who has been convicted in a court of competent jurisdiction of violating the Fish and Game Code, Title 14 regulations, or any other state or federal statute or regulation pertaining to wildlife or animal cruelty, the suspension or revocation shall take effect when the permittee receives a notice of suspension or revocation. The permittee may submit a written request to the commission for a hearing to show cause why his/her permit should be reinstated.

(B) For a permittee who has violated the Fish and Game Code, Title 14 regulations, any term or condition of a commercial native rattlesnake permit, or any other state or federal statute or regulation pertaining to wildlife or animal cruelty, but has not been convicted of any such violation, the suspension or revocation shall not take effect unless 15 calendar days have passed from the date the permittee receives an accusation sent pursuant to Government Code Section 11503, and the permittee has not submitted to the commission a notice of defense described in Government Code Section 11506. If a permittee submits a timely notice of defense, the suspension or revocation shall take effect if, after a commission hearing, the commission finds by a preponderance of evidence that the department's suspension or revocation is warranted.

(f) Humane Care and Treatment. Permitted facilities that house live native rattlesnakes shall comply with the following provisions:

(1) Enclosures. The perimeter of the enclosure for snakes 33 inches in length or less shall be 1.5 times the length of the snake. The perimeter of the enclosure for snakes more than 33 inches in length shall be 1.25 times the length of the snake. The perimeter shall be measured on the inside of the top edge of the enclosure. Snakes may be kept in smaller cages or containers for 31 calendar days from the date of birth or hatching and while being transported. All enclosures shall be adequately ventilated. The substrate shall facilitate the ability to maintain a clean and healthy environment for each animal.

(2) Food. Food shall be wholesome, palatable and free from contamination and shall be supplied in sufficient quantity and nutritive value to maintain the animal in good health.
(3) Water. Potable water shall be accessible to the animals at all times or provided as often as necessary for the health and comfort of the animal. All water receptacles shall be clean and sanitary.
(4) Cleaning of enclosures. Excrement shall be removed from enclosures as often as necessary to maintain animals in a healthy condition.
(5) Disinfection of enclosures. All enclosures shall be disinfected after an animal with an infectious or transmissible disease is removed from an enclosure.
(6) Pest control. Programs of disease prevention and parasite control, euthanasia and adequate veterinary care shall be established and maintained by the permittee.
(7) Observation. Animals shall be observed at least twice a week by the permittee or once a week if the animals are in hibernation. Sick, diseased, stressed, or injured animals shall be provided with care consistent with standards and procedures used by veterinarians or humanely destroyed.
(8) Handling. Animals shall be handled carefully so as not to cause unnecessary discomfort, behavioral stress, or physical harm to the animal.

(g) Emergency Action Plan.

(1) Every commercial native rattlesnake permittee that houses live native rattlesnakes shall have a written Emergency Action Plan readily available, posted in a conspicuous place, and shall submit a copy to the department with the initial permit and renewal application. The Emergency Action Plan shall be titled, with a revision date, updated annually and include, but is not limited to the following:
(A) List of the re-capture equipment available;
(B) Description of humane lethal dispatch methods and a list of qualified personnel who are trained to carry out the methods;
(C) List of medical supplies/first aid kits and where they are located;
(D) Description of mobile transport cages and equipment on hand;
(E) List of emergency telephone numbers that includes the local department regional office, 911, and animal control agencies; and
(F) Written plan of action for emergencies to include but not be limited to rattlesnake bites, escape of rattlesnakes, and emergency facility evacuations.
(2) Permittees are responsible for the capture, and for the costs incurred by the department related to capture or elimination of the threat, of an escaped rattlesnake or the use of humane lethal force required to capture a rattlesnake that escapes.
(3) Any incident involving a rattlesnake held under a commercial native rattlesnake permit that results in serious injury or death to a person shall be reported immediately to the nearest department regional office. If the department determines that serious injury or death has occurred as a result of contact with a rattlesnake, the permit may be reviewed and subject to change by the department. Additional conditions to the permit may be added at any time to provide for public health and safety.
(4) Permittees shall immediately report by telephone the escape of a rattlesnake possessed pursuant to this section to the nearest department regional office and the nearest law enforcement agency of the city or county in which the rattlesnake escaped.

(h) Records. Every permittee that houses live native rattlesnakes shall keep accurate

accounting records for three (3) years from most recent issuance or renewal of the permit in which all of the following shall be recorded:
(1) The complete scientific name and number of all native rattlesnakes purchased, propagated, transferred, exchanged, died and possessed.
(2) The person from whom the native rattlesnakes were purchased, exchanged or transferred.
(3) The date that the native rattlesnakes were purchased, exchanged, transferred, propagated or died.
(4) All required records shall be legible and in the English language and maintained within the State of California.
(i) Annual Reporting Requirement. No permit shall be renewed unless the permittee submits the record specified in Section 703, on or before December 31 of each year. The permittee must submit the record even if there is zero activity to report, or the permittee is not going to renew the permit.
(j) Shipments. All deliveries or shipments of live native rattlesnakes taken under authority of this section shall have a legible copy of the valid permit attached to the outside of the shipping container, which shall be conspicuously labeled: "Live Rattlesnakes - Handle With Care". This subsection does not supersede any federal, state, or local law or regulation or shipper's requirements concerning shipment of live rattlesnakes.

Note: Authority cited: Sections ~~200, 202, 205, 210, 219 and 2205~~5061, Fish and Game Code. Penal Code 597. Government Code Sections 11503 and 11506. Reference: Sections ~~200-202, 205, 206, 210, 215, 219 and 2205~~5060 and 5061, Fish and Game Code. Penal Code 597. Government Code Sections 11503 and 11506.

Subsection (c) of Section 43, Title 14, CCR, is amended to read as follows:

§ 43. Captive Propagation and Commercialization of Native Reptiles.

… No proposed changes to subsections (a) and (b)

(c) Propagation and Possession for Commercial Purposes. Native reptiles may not be sold, possessed, transported, imported, exported or propagated for commercial purposes, except as provided in ~~Section 40(f), and except~~sections 40(f) and 42 and as follows:

… No proposed changes to subsections (c)(1), (c)(2), and (d) through (k)

Note: Authority cited: Sections 200, ~~202,~~ 205, ~~220,~~265, 5061 and 6896, Fish and Game Code. Reference: Sections 200, ~~202,~~ 205, ~~220,~~265, 5061 and 6896, Fish and Game Code.

Subsection (a) of Section 651, Title 14, CCR, is amended to read as follows:

§ 651. Commercial Take of Native Reptiles and Amphibians for Scientific or Educational Institutions.
(a) Native reptiles and amphibians may be sold to scientific or educational institutions only by owners of biological supply houses who have been issued a permit by the department for such purposes. Persons who hold a valid commercial native rattlesnake permit pursuant to Section 42 or commercial developers of biomedical and therapeutic agents shall be considered scientific and educational institutions for the purposes of this section.

… No proposed changes to subsections (a)(1), (a)(2), (a)(3), and (b) through (i)

Note: Authority cited: Sections 1002, 5061, 6851 and 6896, Fish and Game Code. Reference: Sections 1002, 5050, 5060, 5061, 6850, 6852, ~~6854~~ 6854, 6855, 6895 and 6896, Fish and Game Code.

Subsection (a)(2) of Section 703, Title 14, CCR is added as follows:

§ 703. Miscellaneous Applications, Tags, Seals, Licenses, Permits, and Fees.
(a) Applications, Forms and Fees for January 1 through December 31 (Calendar Year).

…No proposed changes to subsection (a)(1))

(2) Commercial Permit for Native Rattlesnakes
(A) 2018 Commercial Native Rattlesnake Permit Application, DFW 1044 (NEW 4/2017), incorporated by reference herein.

1.	Commercial Native Rattlesnake Permit Fee (New)	$ 208.50
2.	Commercial Native Rattlesnake Permit Fee (Renewal)	$ 113.00
3.	Fee for one initial inspection per facility	$ 606.50

(B) Commercial Native Rattlesnake Permit Record, DFW 1044A (NEW 4/2017), incorporated by reference herein.

…No proposed changes to subsections (a)(3) and (b)

Note: Authority cited: Sections 713, 1002, 1050, 1053, 1745, 2118, 2120, 2122, 2150, 2150.2 ~~and 2157~~, 2157 and 5060, Fish and Game Code. Reference: Sections 395, 396, 398, 713, 1050, 1053, 1745, 2116, 2116.5, 2117, 2118, 2120, 2125, 2150, 2150.2, 2150.4, 2151, 2157, 2190, 2193, 2271, 3005.5, 3007, 3503, 3503.5, 3511, 3513, 3950, 5060, 5061, 10500, 12000 and 12002, Fish and Game Code; and Title 50, Code of Federal Regulations, Parts 21.29 and 21.30.

Comparison of the protective effect of a commercially available western diamondback rattlesnake toxoid vaccine for dogs against envenomation of mice with western diamondback rattlesnake (*Crotalus atrox*), northern Pacific rattlesnake (*Crotalus oreganus oreganus*), and southern Pacific rattlesnake (*Crotalus oreganus helleri*) venom

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OBJECTIVE

To evaluate effectiveness of a commercially available toxoid manufactured from western diamondback (WD) rattlesnake (*Crotalus atrox*) venom against envenomation of mice with WD, northern Pacific (NP) rattlesnake (*Crotalus oreganus oreganus*), and southern Pacific (SP) rattlesnake (*Crotalus oreganus helleri*) venom.

ANIMALS

90 specific pathogen–free female mice.

PROCEDURES

Mice were allocated into 3 cohorts (30 mice/cohort). Mice received SC injections of *C atrox* toxoid (CAT) vaccine (n = 15/group) or adjuvant (15/group) at day 0 and again at 4 weeks. At 8 weeks, mice were challenge-exposed with 1 of 3 venoms. Survival until 48 hours was evaluated by use of log-rank analysis of survival curves and the *z* test for proportions.

RESULTS

6 of 15 WD-challenged CAT-vaccinated mice, 3 of 15 NP-challenged CAT-vaccinated mice, and 0 of 15 SP-challenged CAT-vaccinated mice survived until 48 hours. All adjuvant-only vaccinates survived ≤ 21 hours. Mean survival time of CAT vaccinates was longer than that of adjuvant-only vaccinates for all venoms (1,311 vs 368 minutes for WD, 842 vs 284 minutes for NP, and 697 vs 585 minutes for SP). Results of the *z* test indicated a significantly increased survival rate for vaccinates exposed to WD rattlesnake venom but not for vaccinates exposed to NP or SP rattlesnake venom. Log-rank analysis revealed a significant difference between survival curves of vaccinated versus unvaccinated mice exposed to NP but not WD or SP venom.

CONCLUSIONS AND CLINICAL RELEVANCE

CAT vaccination improved survival rate and survival time after challenge exposure with WD rattlesnake venom and may offer limited protection against NP rattlesnake venom but did not provide significant cross-protection against SP rattlesnake venom. (*Am J Vet Res* 2015;76:272–279)

In 2011, 5,700 incidents of snake envenomation in humans were reported by the American Association of Poison Control Hotlines.[1] The true number of envenomations likely is higher because reporting is not mandatory, many snakebites go unreported, some snake-bite victims do not seek treatment, and some treating physicians do not consult with a poison control center.[2,3] Although the incidence of rattlesnake envenomation in the pet population has not been quantified, it is thought to exceed that for humans (> 150,000 bites/y by 1 estimate[4]) because of a high rate of outdoor exposure, unreported or unnoticed incidents, and a presumed limited-threat judgment for bitten animals.[4,5]

A conditionally licensed WD rattlesnake (*Crotalus atrox*) toxoid vaccine is available for administration to dogs and horses at risk for snakebite and is intended to aid in the reduction of morbidity and deaths attributable to rattlesnake envenomation.[6,7] The authors are not aware of any data on evaluation of the effectiveness of the CAT vaccine in scientific journals.[8] Manufacturer data and advertisements suggest this CAT vaccine is efficacious against bites from WD rattlesnakes and also provides cross-protection against envenomation from other rattlesnake species.[9,a] However, analysis of snake venom reveals it to be a complex milieu of peptides and proteins, and venom from related species and subspecies of rattlesnakes can differ markedly in composition.[10-13] A vaccine that

ABBREVIATIONS

ADE	Antibody-dependent enhancement
CAT	*Crotalus atrox* toxoid
NP	Northern Pacific
OD	Optical density
SP	Southern Pacific
WD	Western diamondback

comprises venom from a single species might provide only limited protection against envenomation by other species of rattlesnakes. In California, companion animals are not typically exposed to WD rattlesnakes because these rattlesnakes are found only in sparsely populated areas in the southeast region of the state. Rather, pets are much more likely to encounter NP rattlesnakes (*Crotalus oreganus oreganus*) and SP rattlesnakes (*Crotalus oreganus helleri*), which inhabit heavily populated and traversed regions of central and coastal California. Therefore, we hypothesized that the CAT vaccine might provide limited cross-protection against 2 important species of rattlesnakes found in California. The purpose of the study reported here was to use rattlesnake envenomation of mice to evaluate the comparative effectiveness of the CAT vaccine against the venom of WD, NP, and SP rattlesnakes.

Materials and Methods

ANIMALS

Ninety specific pathogen–free outbred female Swiss Webster mice (4 to 6 weeks old) were obtained from a commercial source. Mice were allowed to acclimate for 72 hours. Mice were housed in groups (5 mice/cage) on corncob bedding with cotton nesting material in individually ventilated cages in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited biocontainment facility. All mice were fed standard laboratory rodent chow and provided with ad libitum access to reverse-osmosis-purified acidified water. The room was maintained at 20° to 21°C with relative humidity of 30% to 70%, 10 to 15 air changes/h, and a photoperiod of 12 hours of light to 12 hours of darkness. Use of the mice in this study was approved by the Institutional Animal Care and Use Committee of the University of California-Los Angeles.

EXPERIMENTAL PROCEDURES

A randomized, blinded, placebo-controlled study was conducted. On the basis of an a priori power analysis (power = 0.8, 0% censoring, and 50-to-50 ratio of control mice to experimental mice), the 90 mice were randomly selected by an individual unaffiliated with the study and assigned to treatment and control groups (45 mice/group). Treatment mice received an injection (0.2 mL, SC) of CAT vaccine[b] at day 0 and again at 4 weeks. Control mice received an injection (0.2 mL, SC) of pharmaceutical-grade aluminum hydroxide adjuvant[c] at day 0 and again at 4 weeks. Four weeks after administration of the second injection of CAT vaccine or adjuvant, mice were challenge-exposed with rattlesnake venom.

VENOM

The Society for the Study of Amphibians and Reptiles classification of the western rattlesnake (*Crotalus oreganus*) was used for the present study. The NP and SP rattlesnakes are 2 of 5 recognized subspecies of western rattlesnake, and the WD rattlesnake is a monotypic species with no recognized subspecies. Lyophilized WD rattlesnake venom was obtained.[d] The venom was collected from WD rattlesnakes throughout the range of these rattlesnakes within the United States. Venom of NP and SP rattlesnakes was collected from various regions throughout northern and southern California[14–16] **(Figure 1)**. Samples of NP rattlesnake venom were collected at Sanger (Fresno County), Sutter Butte (Sutter County), Lake Berryessa (Napa County), Vacaville (Solano County), Johnsondale (Tulare County), and Modesto (Stanislaus County). Samples of SP rattlesnake venom were collected at Rasnow Peak, Hidden Valley, Santa Rosa Valley, Carlisle Canyon, Lake Sherwood, and Oak Park (Ventura County); Acton, Castaic, Leona Valley, Topanga Canyon, Malibu Canyon, and Griffith Park (Los Angeles County); Oak Hills, Phelan, Devil's Canyon, and Big Bear (San Bernardino County); Idyllwild-Pine Cove and Garner Valley (Riverside County); and De Luz (San Diego County). Venom samples were processed in accordance with a standardized protocol. The final lyophilized venom product contained equal parts (vol/vol) from each sample location. In preliminary experiments, the LD_{50} was estimated for each venom on the basis of the animal-sparing up-and-down LD_{50} testing paradigm.[17–26] Those LD_{50} values then were used in the study as follows: WD rattlesnake venom, 2.8 mg/kg; NP rattlesnake venom, 1.7 mg/kg; and SP rattlesnake venom, 1.5 mg/kg. These LD_{50} values are similar to those published previously.[27–31]

Figure 1—Map of the distribution for WD rattlesnakes (*Crotalus atrox*; black-shaded area), NP rattlesnakes (*Crotalus oreganus oreganus*; light gray–shaded area), and SP rattlesnakes (*Crotalus oreganus helleri*; dark gray–shaded area) in California and locations for collection of venom samples (circles). The range of each of the rattlesnakes was obtained from previously published information.[14–16] Notice that major metropolitan population centers are located exclusively in the ranges of NP and SP rattlesnakes.

Table 1—Summary of survival data for mice inoculated with CAT vaccine or adjuvant only at 0 and 4 weeks and challenge-exposed 4 weeks later with venom of WD rattlesnakes (*Crotalus atrox*), NP rattlesnakes (*Crotalus oreganus oreganus*), and SP rattlesnakes (*Crotalus oreganus helleri*).

	WD rattlesnake venom		NP rattlesnake venom		SP rattlesnake venom	
Variable	**Vaccine**	**Adjuvant only**	**Vaccine**	**Adjuvant only**	**Vaccine**	**Adjuvant only**
No. of mice injected with venom	15	15	15	15	15	15
No. of mice that survived to 48 h after venom injection	6	0	3	0	0	0
Survival time (min)						
Mean	1,311	368	842	284	697	585
Minimum	121	238	82	160	295	114
Maximum*	2,880	422	2,880	401	1,440	1,269
P value†						
z test for proportions	0.006		0.068		—	
Log-rank analysis	0.146		0.010		0.166	

*An endpoint of 2,880 min (ie, 48 hours) for survival was determined prior to the study (ie, surviving mice were euthanized at 48 hours after venom injection). Despite the fact some mice were expected to live > 48 hours after venom injection, survival time was limited in this manner to avoid effects on reported mean survival times in surviving mice and is in accordance with commonly accepted practices for survival studies.[23] †Values were significant at $P \leq 0.05$.

— = Not applicable because there were no surviving mice in either of these groups.

VENOM CHALLENGE EXPOSURE

Three cohorts (30 mice/cohort [15 treated mice and 15 control mice]) were challenge-exposed with 1 of the 3 venoms at 4 weeks after the second injection of CAT vaccine or adjuvant. Venom was administered to each mouse via IP injection at twice the calculated LD_{50}. For injection, lyophilized venom was reconstituted in sterile water to create a stock solution of 5 mg/mL, which was then diluted as needed to provide the dose for administration. Mice were closely monitored for 48 hours after venom administration.

Before venom administration, body weight and baseline core body temperature were recorded. Temperature was obtained with a 1.5-cm-long thermistor probe inserted via the rectum into the colon; temperature was recorded once per hour for up to 10 hours and thereafter as needed. An observer who was unaware of the venom administered or vaccination status of the mice assessed their condition and determined when a mouse would be euthanized. Mice were euthanized by gradual-fill CO_2 inhalation when they became nonresponsive to stimuli, were in marked respiratory distress (agonal breathing or intermittent gasping), or had a prolonged period of moribundity (severely limited response to stimuli and core body temperature < 70% of the baseline core temperature for > 2 hours). Surviving mice were euthanized 48 hours after venom administration, and a postmortem blood sample was obtained via cardiocentesis.

ANTIBODY TITERS

Blood samples were collected from the retroorbital venous sinus of isoflurane-anesthetized mice 1 week before venom challenge exposure (ie, 3 weeks after the second injection of CAT vaccine or adjuvant) for use in determination of 2 sets of serum antibody titers. First, to verify that mice generated antibodies against the CAT vaccine, serial serum antibody titers of 3 randomly selected vaccinated mice were compared with serial serum antibody titers of 3 randomly selected adjuvant-only control mice. Second, to compare specificity of antibodies generated, dilutions (1:8,000) of serum obtained from 8 randomly selected vaccinated mice were tested against each of the 3 venoms. To generate serial titers and evaluate antibody specificity, 96-well ELISA plates were coated (100 µL/well) with reconstituted venom diluted in 0.1M carbonate buffer (1 µg/mL). Plates were sealed with acetate and incubated overnight at 22°C. After incubation, wells were washed (PBS solution with 0.05% Tween20) and then blocked by incubating on a plate shaker for 15 minutes at 22°C. Diluted serial serum samples were then applied to wells in triplicate. Plates were incubated on a plate shaker for 30 minutes at 22°C. Wells then were washed and horseradish peroxidase–conjugated goat anti-mouse IgG was added; plates were incubated on a plate shaker for 30 minutes at 22°C. Wells were then washed, and the chromogenic substrate tetramethylbenzidine was added. After incubation on a plate shaker for 10 minutes, the reaction was stopped by the addition of 2N sulfuric acid; plates then were immediately evaluated to determine the OD at 450 nm by use of an automated ELISA reader. The OD was used as an indicator of the presence of antivenom IgG as well as for comparisons of relative reactivity between venom types and general assessment of interindividual variation.

STATISTICAL ANALYSIS

Mean survival time in minutes and Kaplan-Meier survival curves were generated for the 3 venoms and saline (0.9% NaCl) solution control samples. A *z* test of proportions was used to compare survival rates of vaccinated versus control mice for all venoms. Log-rank analysis was used to compare Kaplan-Meier survival curves of vaccinated versus control mice for all venoms. Multilevel, mixed-effects linear regression modeling[e] was used to compare specificity of an antibody

titer of 1:8,000 for all venoms. Significance for all tests was set at $P \leq 0.05$.

Results

SURVIVAL RATE AND SURVIVAL TIME

Both survival rate and survival time were analyzed **(Table 1)**. For mice vaccinated with CAT vaccine, 6 of 15 mice challenge-exposed with WD rattlesnake venom, 3 of 15 mice challenge-exposed with NP rattlesnake venom, and 0 of 15 mice challenge-exposed with SP rattlesnake venom were alive at 48 hours after venom injection, whereas adjuvant-only control mice survived ≤ 21 hours after injection of any of the 3 rattlesnake venoms. Mean survival time of vaccinated mice was longer than that of adjuvant-only control mice for all venoms (1,311 vs 368 minutes for WD rattlesnake venom, 842 vs 284 minutes for NP rattlesnake venom, and 697 vs 585 minutes for SP rattlesnake venom). Survival analysis for individual venom revealed that results of the *z* test for proportions were significant ($P = 0.01$) only for WD rattlesnake venom. Log-rank analysis of survival curves revealed significant ($P = 0.01$) differences only for NP rattlesnake venom **(Figure 2)**. Maximum survival time was greatest for vaccinated mice, compared with survival time for adjuvant-only control mice, for all venoms. Notably, minimum survival time was greater for control mice than for vaccinated mice for both WD and NP rattlesnake venoms. This was evident on the Kaplan-Meier survival curve for WD rattlesnake venom as an initial increase in death of vaccinated mice, compared with that of control mice, at early time points (< 300 minutes after venom injection). Because of this finding, a log-rank analysis for WD rattlesnake venom that excluded early time points was conducted (n = 7 mice) and revealed a significant ($P = 0.004$) effect.

Student *t* test analysis of prestudy mean body weight and baseline core body temperature revealed that these variables did not differ significantly among any of the groups ($P = 0.08$ to 0.67; data not shown). No morbidity or deaths were associated with receiving the vaccine or adjuvant alone.

ANTIBODY TITERS

Antibody titers against all 3 rattlesnake venoms for the 3 vaccinated and 3 control mice were plotted **(Figure 3)**. Dilutions tested were 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000, and 1:128,000. Mice vaccinated with CAT developed measurable antibody titers against all 3 venoms, whereas mice receiving only adjuvant had no evidence of reactive serum antibodies against any venom. The OD for a 1:8,000 dilution of serum obtained from 8 additional randomly selected vaccinated mice tested against all 3 venoms was plotted **(Figure 4)**. Comparison of OD for the various venoms suggested a decreasing reactivity as follows: the reactivity of WD rattlesnake venom was greater than that of NP rattlesnake venom, and the reactivity of NP rattlesnake venom was greater than that of SP rattlesnake venom. Analysis of a multilevel mixed-effects linear regression model with venom as the sole categorical predictor revealed significant ($P \leq 0.001$) differences in OD for each venom. Interindividual variation was also evident because the majority (6/8) of the mice had titers with OD values approaching or exceeding 1.0, whereas the remainder (2/8) had OD values < 0.5.

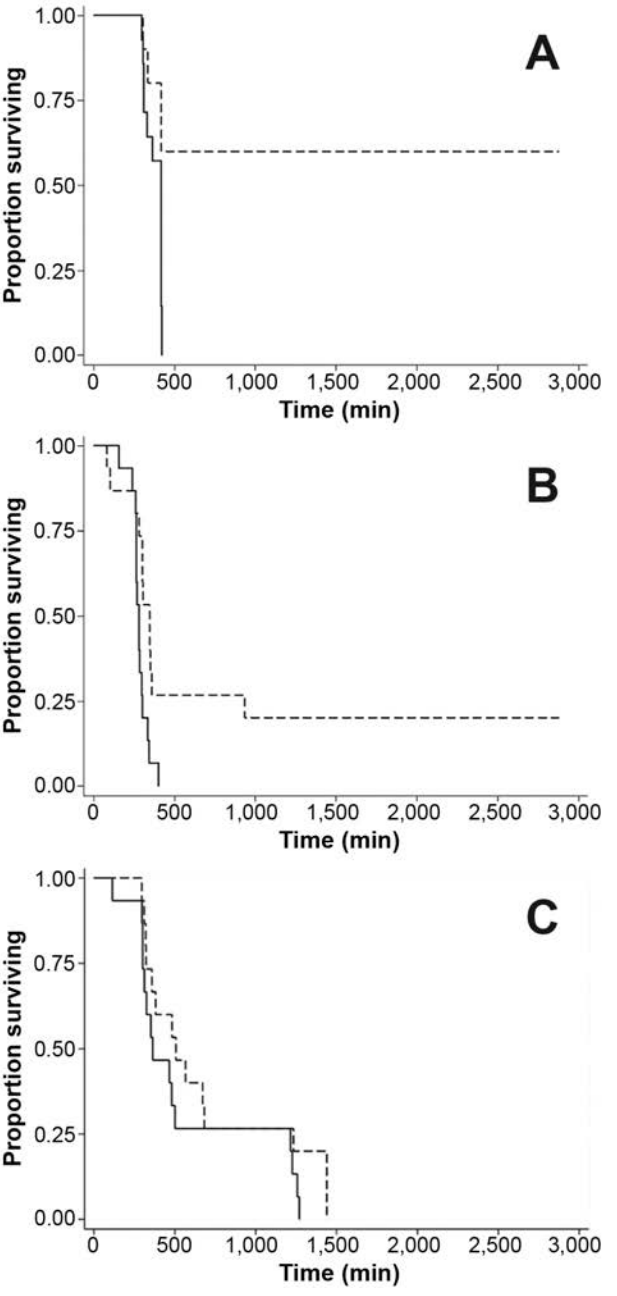

Figure 2—Kaplan-Meier survival curves for vaccinated mice (dashed lines) and adjuvant-only control mice (solid lines) after challenge exposure with WD rattlesnake venom (A), NP rattlesnake venom (B), and SP rattlesnake venom (C). There were 15 mice in each group. Time of challenge exposure (injection of venom) was designated as time 0. There was a significant ($P = 0.01$; log-rank analysis) difference in survival curves of vaccinated versus adjuvant-only mice after injection of only NP rattlesnake venom. In panel A, notice the possible early death phenomenon attributable to ADE of WD rattlesnake venom.

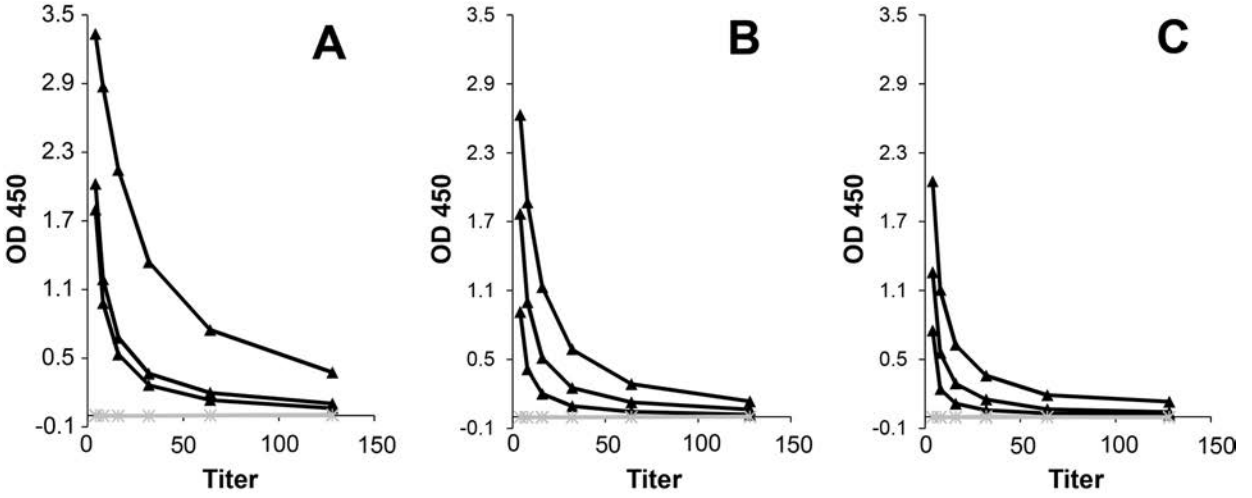

Figure 3—Serial serum dilution antibody titers for 3 vaccinated mice (black symbols) and 3 adjuvant-only control mice (gray symbols) against venom of WD rattlesnakes (A), NP rattlesnakes (B), and SP rattlesnakes (C) as determined by OD measured at 450 nm (OD 450). Each black symbol represents results for 1 mouse; the gray symbol represents results for 3 mice. Notice that the antibody response of vaccinated mice was greater than that of the control mice for all venoms. There was a pattern that specificity (ie, increased OD 450) was greater against venom of WD rattlesnakes than against venom of NP or SP rattlesnakes. The x-axis represents a dilution factor of 1:1,000. Dilutions tested were 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000, and 1:128,000.

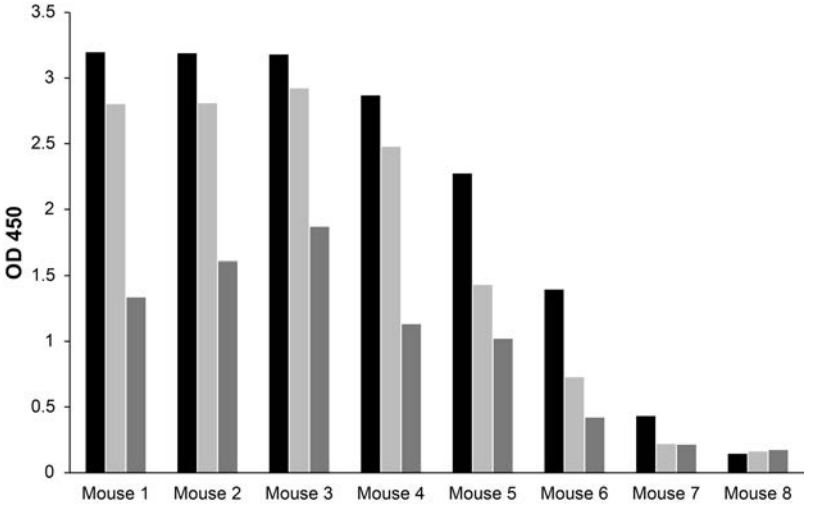

Figure 4—Single serum dilution (1:8,000) antibody titers for 8 randomly selected mice against venom of WD rattlesnakes (black bars), NP rattlesnakes (light gray bars), and SP rattlesnakes (dark gray bars). Notice the marked interindividual differences as well as differences in specificity among venoms (WD rattlesnake > NP rattlesnake > SP rattlesnakes venom). There was a significant ($P \leq 0.001$; multilevel mixed-effects linear regression) difference in OD 450 among venoms.

Discussion

In the present study, survival analysis after rattlesnake envenomation of mice was conducted in a randomized, blinded, placebo-controlled study to evaluate the comparative effectiveness of CAT vaccine against 3 rattlesnake venoms. The data reported included evaluation of survival rate (whether a mouse died ≤ 48 hours after venom injection) as well as evaluation of survival time (number of minutes a mouse survived after venom injection, up to 48 hours). Survival time is an important consideration in light of the fact a venom vaccine may be useful if it extends the course of the envenomation, thereby allowing additional time to seek primary medical treatments such as antivenin and intensive care. In addition, antibody titers of vaccinated and adjuvant-only control mice were compared as well as specificity of the antibodies generated against each of the 3 venoms. Overall, results of the challenge-exposure experiment indicated that CAT vaccination resulted in a significant increase in survival rate and survival time against injection with WD rattlesnake venom; equivocal results after injection of NP rattlesnake venom, which would likely require a greater number of mice to verify a difference; and no significant improvement in survival measures after injection of SP rattlesnake venom. Analysis of antibody titers revealed a clearly measurable antibody response in vaccinated mice, compared with that in adjuvant-only control mice, against all 3 venoms. The antibodies were most reactive against WD rattlesnake venom, with significantly less reactivity against venoms of the 2 other rattlesnake species.

Analysis of the data for the present study indicated that administration of CAT vaccine conferred an increase in survival rate and survival time in vaccinated versus control mice challenge-exposed with WD rattlesnake venom. Mean survival time was greater in vaccinated than in control mice, and survival rate improved significantly ($P = 0.01$; z test for proportions). Unexpectedly, results for log-rank analysis of

survival curves did not reveal significant differences. This result was particularly surprising because challenge exposure with NP rattlesnake venom had a significant effect, as determined by use of log-rank analysis, despite the fact there were only half as many survivors as for challenge exposure with WD rattlesnake venom. Notably, minimum survival time was greater for control versus vaccinated mice for both WD and NP rattlesnake venom (Table 1). This was also evident on the Kaplan-Meier survival curve for WD rattlesnake venom as an initial increase in death of vaccinated versus adjuvant-only control mice at early time points (< 300 minutes after venom injection; Figure 2). The early deaths may have sufficiently altered early time points of the curve of vaccinated mice after injection of WD rattlesnake venom such that statistical modeling resulted in a curve for vaccinated mice that was indiscernible from the curve for the control mice, despite the clear difference at later time points ($P = 0.004$ for log-rank analysis after 300 minutes). We propose that the early deaths could have been attributable to 1 factor or a combination of factors, such as genetic predisposition to venom sensitivity, injection near or into a vascular bed that hastened systemic exposure to venom, or an antibody-mediated early death phenomenon that has been observed in a laboratory setting when testing vaccines against viruses and bacterial toxins.[32–39]

Use of the vaccine may afford limited cross-protection against NP rattlesnake venom; however, the data are not entirely conclusive. Mean survival rate of vaccinated mice significantly (P = 0.01; log-rank analysis of survival curves) exceeded that of adjuvant-only control mice, which suggested a protective effect. However, results of the z test for proportions of survival time did not reveal significant ($P = 0.07$) differences. However, it is plausible that testing a larger population of mice may have allowed us to detect a more subtle effect by use of the z test of proportions.

The vaccine did not provide significant protection against SP rattlesnake venom, although the mice with the greatest survival time were in the vaccinated group. The CAT vaccine may have been less effective against SP rattlesnake venom because of the divergent molecular composition of that venom. For example, 1 population of SP rattlesnakes can produce Mojave toxin, a unique and powerful neurotoxin, which to date has not been found in WD or NP rattlesnake venoms.[15,40]

In addition to survival analysis, antibody titers were measured in a number of mice to verify an antibody response against the CAT vaccine (Figure 3). Compared with control mice, vaccinated mice had a variably robust antibody response, and initial titers suggested that the antibodies were more specific for WD rattlesnake venom than for the NP or SP rattlesnake venoms. On the basis of this observation, sera from 8 randomly selected vaccinated mice were evaluated for antibody specificity against each of the 3 venoms evaluated in the study (Figure 4). Linear regression analysis revealed significantly increased OD against WD rattlesnake venom, as compared with results against SP or NP rattlesnake venoms. The analysis indicated that antibodies generated by mice were most specific against the venom of manufacture (ie, WD rattlesnake venom), compared with specificity against the other 2 genetically distinct venoms. It should be emphasized that antibody titers were measured only to verify that mice generated an antibody response against the vaccine and to evaluate the specificity of that antibody response. The magnitude of the murine antibody response and how it may relate to survival of vaccinated dogs and horses (or the ability of clinicians to provide a prognosis for survival of vaccinated animals) in real-life situations were beyond the scope of the present study.

The present study had several potential confounders. First, on the basis of a previous manufacturer-designed study,[a] mice in the present study were injected with a vaccine dose of 0.2 mL, which could be from 50- to 1,500-fold as high (by volume) as manufacturer-recommended doses for dogs and horses.[6,7] Potentially, this could have resulted in a more robust antibody response and more enhanced protective benefit than typically would be afforded to companion animals. On the other hand, it should be mentioned that mice were challenge-exposed with an extremely high (twice the LD_{50}) dose of venom administered via the IP route commonly used in venom studies on mice. In most naturally occurring scenarios, companion animals receive SC or IM injection of venom, which results in slower and less immediately severe systemic effects[41] than were seen in the mice of the study reported here. In light of this, findings for the present study should be considered with the caveat that, in theory, the vaccine may improve survival rate and survival time, but these improvements remain to be definitively verified in practice settings for the specific species and situations of interest. Finally, it should be mentioned that we evaluated survival rate and survival time but did not directly assess morbidity. In actual envenomations, local effects such as severe necrosis, hemorrhage, and inflammation can cause substantial morbidity, which potentially can lead to severe incapacitation and death.[42–45] It remains to be determined whether vaccination has substantial effects to prevent or reduce important local sequelae after snake envenomation. Despite these drawbacks, there are a number of reasons investigators should use the described method of envenomation of mice, including that it is a well-accepted technique for venom analysis and antivenin evaluation, adheres to the concept of replacement in research (ie, use of mice instead of dogs or horses), and has been used in experiments conducted by the manufacturer to obtain USDA licensing for the CAT vaccine.

Data from the rattlesnake envenomation of mice reported here indicated that administration of the CAT vaccine resulted in a significant increase in survival

rate and survival time after injection of WD rattlesnake venom, equivocal results after injection of NP rattlesnake venom (possibly requiring a greater number of animals to confirm a difference), and no significant improvement in survival variables after injection of SP rattlesnake venom. Analysis of antibody titers confirmed a measurable antibody response in vaccinated versus adjuvant-only control mice and confirmed that specificity of the antibody response was significantly greater against the venom of manufacture. Overall, results of the present study suggested that vaccination with the CAT vaccine may provide limited cross-protection against NP rattlesnake venom but no significant cross-protection against SP rattlesnake venom. Future studies should include more in-depth analysis of antibody titers, testing of alternative vaccination strategies involving other venoms, and investigation into early deaths seen in some of the vaccinated mice. Such studies will be useful in validating results of the present study and providing increased insight into the real-world effectiveness of a rattlesnake venom vaccine.

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Footnotes

a. Taylor J, Moody L, Pollock H, et al. *Crotalus atrox* toxoid elicits a venom neutralizing antibody response in vaccinated dogs (abstr), in *Proceedings*. Western Vet Conf 2005.
b. Rattlesnake vaccine for dogs, serial No. 13-082, US veterinary license No. 407, Red Rocks Biologics, Woodland, Calif.
c. Alhydrogel 2%, lot No. AHG-35-01, InvivoGen Inc, San Diego, Calif.
d. Lot No. CAT 1412, Kentucky Reptile Zoo, Slade, Ky.
e. STATA, version 13, StataCorp, College Station, Tex.

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